

MEDICINE AND DENTISTRY

1. Introduction

Nowadays the association between gestational parvovirus B19 infection and fetal loss has increased interest in this virus and demand for additional desirably noninvasive diagnostic procedures. This human pathogenic virus associated with a wide range of clinical conditions, may affect many fetal organs and can cause fetal morbidity and mortality with severe anemia, following fetal erythroid progenitor cells infection and apoptosis. Severe anemia may cause high output cardiac failure and nonimmune fetal hydrops. In addition, parvovirus B19 may directly infect myocardial cells and produce myocarditis that further aggravates the cardiac failure [1, 2]. So, improvement of new practical and reliable methods for identification fetal abnormalities are becoming more important in the modern obstetrics.

Maternal serum alpha-fetoprotein testing is commonly used during the second trimester to screen for various fetal genetic defects (neural tube defects, chromosomal abnormalities). However, its role in anemia and erythropoiesis is less recognized. Levels of this biochemical marker can be elevated in women infected by parvovirus B19 and probably come of from damage fetal liver cells or infected placental cells [3], but there is still no clear data on this fact, which is particularly relevant. Maternal alpha-fetoprotein is correlated with both Doppler blood flow velocity parameters in the middle cerebral artery and fetal hemoglobin. Elevations of this marker may appear earlier than Doppler abnormalities in cases of fetal anemia associated with hydrops [3].

Also routine histological studies can play an important role in the diagnosis of B19 infection and provide a strong presumptive evidence of intrauterine fetal injure.

PLACENTAL CYTOPATHIC DAMAGE FACTOR DUE TO PARVOVIRUS B19 – AS A HALLMARK OF INTRAUTERINE SYMPTOMATHY OF INFECTED FETUS

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Abstract: Aim of the work. The focus of this study was to analyze the frequency of fetal intrauterine symptomatic realization as a result of B19 parvovirus infection of pregnant women during I and II gestation periods, with the combination of determination the levels of alpha-fetoprotein in maternal serum blood and histopathological investigation of placenta in cases of negative effects for infected fetuses.

Materials and methods. A total of 478 pregnant women were at risk of infection and screened for parvovirus B19. The study was based on serological, morphological and virological testing of fetuses from mothers with confirmed parvovirus B19 infection. Infection of pregnant women was detected by serological diagnosis of specific antibodies IgM and IgG to parvovirus B19. Intrauterine fetal infection was confirmed by polymerase chain reaction method. Levels of alpha-fetoprotein in maternal venous blood were detected using an immunochemical test method. An antenatal diagnosis of hydrops fetalis was confirmed by ultrasound scanning. Placenta from the 8 infected fetuses was studied histologically with hematoxylin and eosin staining using electron microscope during gestational periods of 12–22 weeks. Statistical analysis of the obtained data was carried out using nonparametric statistics with the definition of Fisher's criterion.

Results and discussion. The average gestational period of symptomatic manifestations of intrauterine fetal infection was 19.9 ± 0.5 weeks of gestation. It was established that in 33 of cases were confirmed the intrauterine infection due to parvovirus B19 by using the morphological placenta tissue analysis. The research found that the levels of maternal serum alpha-fetoprotein was significantly higher (in 2.17 times) in group of fetuses who suffered from fetal hydrops and in cases of intrauterine fetal death before detecting the general echographic sings of fetal failure ($p < 0.05$). It will be very important to compare this indicator with the Doppler ultrasound measurements of the human fetal middle cerebral artery peak systolic velocity (MCA PSV) and blood flow parameters in the fetal ductus venosus and to predict the possibility of poor perinatal outcomes. It was identified a cytopathic effect in placental cells viral origin and detected in cells of the cytotrophoblast, syncytiotrophoblast, endothelial cells, and blood cells.

Conclusions. Parvovirus B19 fractions were visualized in 100 % of cases of miscarriages with fetal hydrops and placental edema during 12–22 weeks of gestation, while acute maternal parvovirus infection (with the detection of IgM against to parvovirus B19) was diagnosed by immunoassay only in 62.5 % of cases.

Detection of B19 particles in placental tissue from fetuses with non-immune origin hydrops is an auxiliary method in the diagnosis of vertical transmission of parvovirus during pregnancy.

It is more preferable to estimate the levels of alpha-fetoprotein in maternal serum blood in case of parvovirus B19 affected pregnancies as raising rates of this marker may be an early prediction hallmark for adverse fetal outcome.

Keywords: histopathology, parvovirus B19 infection, B19 virions, non-immune fetal hydrops, fetal death, spontaneous miscarriage, cytotrophoblast, syncytiotrophoblast, pregnancy, intrauterine symptomathy.

The susceptibility of placental cells – cytotrophoblast and syncytiotrophoblast to parvovirus B19 infection and its cytotoxic effect on target cells determine the nature of the pathogenic disorders, including spontaneous miscarriages, stillbirth and fetal death before full 21 gestation weeks. Numerous clinical studies quite clearly show that the most of women who were infected with parvovirus during pregnancy gave birth to healthy children [4]. According to the literature data, the most negative effect for the fetus and risk of intrauterine fetal death is the largest when the parvovirus B19 infection appeared before 20 weeks of pregnancy. These terms are corresponding to the development and formation of the placenta – critical period of placentation. Transmission of virus may occur not only through damage in the protective placental barrier, but also through endocytosis or receptor-mediated transfer. The mechanism by which the virus can be transmitted from the infected mother to the fetus was studied using experimental models which found that immunoreactivity for B19 infection was most pronounced in the sponged trophoblastic placental cells in the first trimester; reduction in reactivity was observed in the placenta cells in the second trimester, but almost no coloration of the antigen was noted in the third trimester [5]. The reason for this difference for fetal effects is not only the presence of a greater number of target cells during the first and second trimester, but also a greater number of viral receptors on the spider trophoblast layer of the placenta. Infection with pregnant parvovirus B19 is usually diagnosed using the immune enzyme method to detect IgM antibodies to parvovirus [6]. According to statistical data, vertical transmission of parvovirus to the

fetus occurs in 56 % of cases. However, fetal hydrops may occur, as in the symptomatic and asymptomatic clinical maternal forms of parvovirus infection [7, 8]. In serum specific antibodies to B19 can disappear within 3–4–5 weeks from the onset of acute infection [9]. In such cases, high levels of IgG to parvovirus may indicate a history of a mother's infection. Thus, testing for parvovirus-specific IgM before childbirth or miscarriage may be ineffective in terms of diagnosing intrauterine infection [10]. More informative diagnostic hallmark for parvovirus infection is the detection viral DNA fraction in the mother's serum blood [11] by means of a nested polymerase chain reaction of dot-blot hybridization and hybridization in situ.

Aim of the research was to analyze the frequency of fetal intrauterine symptomatic realization as a result of B19 parvovirus infection of pregnant women during I and II gestation periods, with the combination of determination the levels of alpha-fetoprotein in maternal serum blood and histopathological investigation of placenta in cases of negative effects for infected fetuses.

2. Methods

The study was conducted during the period from January 2011 to May 2018 and based on serological, morphological and virological testing of fetuses from mothers with confirmed parvovirus B19 infection. Infection of pregnant women was detected by serological diagnosis of specific antibodies IgM and IgG to parvovirus B19.

Intrauterine fetal infection was confirmed by polymerase chain reaction (PCR) reagents with hybridization detection «AmpliSens» (Russia) and the immune enzyme immunoassay method, the test system EUROIMMUN (Germany).

Levels of alpha-fetoprotein in maternal venous blood were detected using an immunochemical test method with electrochemiluminescence immunoassay (ECLIA) on Cobas 6000 (e 601 module), test system Roche Diagnostics (Switzerland).

An antenatal diagnosis of hydrops fetalis was confirmed by ultrasound scanning.

Placenta from the 8 infected fetuses was studied histologically with hematoxylin and eosin staining according to standard procedures during gestational periods of 12–22 weeks. The material was fixed in 2.5 % glutaraldehyde solution on phosphate buffer with fixation with 1 % solution of osmium tetroxide. The material dehydrated in alcohols with increasing concentration (70 %, 80 %, 90 %, 100 %) and with the help of acetone. After block polymerization methods we used ultramicrotomes LKB III (Sweden) and Reihart (Austria), ultrathin sections of 40 nm were made which were contrasted with a 2 % solution of uranyl acetate and lead citrate. The microslides were studied and photographed under an electron microscope of PEM-125K with an increase of 9.6–20 thousand times. Viruses were studied in microphotographs with an increase of 40–60 thousand. Statistical analysis of the obtained data was carried out using nonparametric statistics with the definition of Fisher's criterion.

3. Results

A total of 478 pregnant women were at risk of infection and screened for parvovirus B19. During serological and virological testing, it was found that 32 % of women had already immunized against parvovirus B19, that is, they have had in the past B19. In the serum of these pregnant women, only specific IgG antibodies to parvovirus B19 were determined. 41 % of pregnant women had negative results of laboratory diagnosis of parvovirus, which indicates that they had never met parvovirus B19 before. 26.9 % of women had positive indica-

tors of virological and serological markers of virus, they were included the main research group, at one time they were subdivided according to gestational periods and symptomatic manifestations of parvovirus infection (1st trimester – 45 women, 2nd trimester – 48 women). The average gestational period of symptomatic manifestation of intrauterine fetal infection was 19.9 ± 0.5 weeks of gestation. In 33 cases we confirmed the intrauterine infection due to parvovirus B19 (testing of placenta tissue). The study revealed the distribution of the main group of pregnant women according to symptomatic manifestations: in the first trimester it was found: 13.3 % of women had asymptomatic course of parvovirus B19 infection during the examination; with moderate and severe women symptoms during of pregnancy period – 66.6 % and in the presence of typical symptoms of the fetus (hydrops, cardiomegaly, fetal growth retardation, congenital malformation, calcinosis in the liver, spleen, pericardium, severe fetal anemia, etc.) – 20.1 % of women. Compared with the 2nd trimester, the asymptomatic course of the infection and symptomatic course for the fetus had a more pronounced character: asymptomatic – in 39.9 % of women compared with the symptomatic maternal course during pregnancy – 23 % and severe fetal symptoms were identified in – 37.5 % of pregnant women.

Histopathology of the placenta showed a strong interstitial and perivascular lymphocytic inflammatory cell infiltrates, accompanied by necrosis, fiber breaks and edema. Electron microscopy examination of placenta from women with negative effects for the fetus infected with parvovirus B19 showed the presence of small amount of viral particles in the nucleus and in the cytoplasm in all studied specimens. To a greater extent, determined a cytopathic effect in placental cells due to a viral infection that was detected in cells of the cytotrophoblast, syncytiotrophoblast, endothelial cells, and blood cells. It reflected the state of the cells which infected by the virus and was determined as the first stages of autophagy. So, in the cytoplasm of these cells, autophagosomes with a double membrane surrounding cell detritus, or virions and their capsids in the form of bubbles, were detected. It is known that autophagosomes absorb long-lived proteins, damaged organelles and even invasive pathogens.

It was interested to notice that in fetuses with fetal hydrops and poor perinatal outcomes (intrauterine fetal death, stillbirth) levels of maternal serum alpha-fetoprotein was significantly higher (in 2, 17 times) before detecting the general echographic sings of fetal failure ($p < 0.05$). It appears to be a prognostic marker for poor pregnancy outcome in case of parvovirus B19 infected pregnancies and should be regularly monitored on the early stages of fetal insufficiency. It will be very important to compare this indicator with the Doppler blood flow velocity parameters in the middle cerebral artery and in the fetal ductus venosus with calculation of peripheral vascular resistance indices and to predict the possibility of poor perinatal outcomes.

Parvovirus B19 fractions were visualized in 100 % of cases of unauthorized termination of pregnancy with fetal hydrops and placental edema in the 12–22 weeks of gestation, while acute parvovirus infection in the mother (with the detection of IgM against to parvovirus B19) was diagnosed by immunoassay only in 62.5 % of cases.

4. Discussion

19-infected erythroblast cells with intranuclear inclusions have been observed in placenta and fetal tissue by many authors with histology and electron microscopy methods [1, 3, 5]. In our research the histopathological assessment of placenta

in case of parvovirus infection is characterized by pronounced placentitis with edema due to the cytolytic action of viruses in the core of endothelial cells of the blood capillaries, as well as in the cytoplasm, myelin-like structures, which contributes to the enhancement of lipid peroxide oxidation. According to research data of RF Lamont and co-authors placentitis may cause placental dysfunction and adverse fetal outcome [12]. The cytopathic effect of infection, which we had established in placental cells was determined as the first stages of autophagy, one of the types of death of infected cells. Detection of particles of parvovirus B19 in placental tissue of fetuses, who suffered from non-immune origin hydrops is an auxiliary method in the diagnosis of vertical transmission of parvovirus B19 during pregnancy.

According to recent data of Y. Ling and co-authors [13] for fetal diseases, maternal alpha-fetoprotein is significantly correlated with fetal hemoglobin and fetal middle cerebral artery blood flow velocity in fetal anemic disease such as red blood cell alloimmunisation and parvovirus infection; it was also reported that this parameter increases earlier than the increase in MCA velocity. Analyzing the received data, we can state that in case of parvovirus B19 affected pregnancies its more preferable to estimate the levels of alpha-fetoprotein in maternal serum blood as raising levels of this marker may be an early prediction hallmark for poor fetal outcome and compare the results of alpha-fetoprotein with the Doppler blood flow velocity parameters in the middle cerebral artery, and in the fetal ductus venosus with calculation of peripheral vascular resistance indices.

References

1. Ornoy, A., Ergaz, Z. (2017). Parvovirus B19 infection during pregnancy and risks to the fetus. *Birth Defects Research*, 109 (5), 311–323. doi: <http://doi.org/10.1002/bdra.23588>
2. Bonvicini, F., Bua, G., Gallinella, G. (2017). Parvovirus B19 infection in pregnancy – awareness and opportunities. *Current Opinion in Virology*, 27, 8–14. doi: <http://doi.org/10.1016/j.coviro.2017.10.003>
3. Chen, C.-C., Chen, C.-S., Wang, W.-Y., Ma, J.-S., Shu, H.-F., Fan, F. S. (2015). Parvovirus B19 infection presenting with severe erythroid aplastic crisis during pregnancy in a woman with autoimmune hemolytic anemia and alpha-thalassemia trait: a case report. *Journal of Medical Case Reports*, 9 (1). doi: <http://doi.org/10.1186/s13256-015-0542-7>
4. Waring, G. J. (2018). Parvovirus B19 infection: Timely diagnosis in pregnancy essential. *Case Reports in Women's Health*, 18, e00057. doi: <http://doi.org/10.1016/j.crwh.2018.e00057>
5. León-Juárez, M., Martínez-Castillo, M., González-García, L. D., Helguera-Repetto, A. C., Zaga-Clavellina, V., García-Cordero, J. et. al. (2017). Cellular and molecular mechanisms of viral infection in the human placenta. *Pathogens and Disease*, 75 (7). doi: <http://doi.org/10.1093/femspd/ftx093>
6. Faucette, A. N., Pawlitz, M. D., Pei, B., Yao, F., Chen, K. (2015). Immunization of pregnant women: Future of early infant protection. *Human Vaccines & Immunotherapeutics*, 11 (11), 2549–2555. doi: <http://doi.org/10.1080/21645515.2015.1070984>
7. Gallinella, G. (2017). Parvovirus B19: recent insights and implications for pathogenesis, diagnosis and therapy. *Microbiologia Medica*, 32 (3). doi: <http://doi.org/10.4081/mm.2017.7109>
8. Shabani, Z., Esghaei, M., Keyvani, H., Shabani, F., Sarmadi, F., Mollaie, H., Monavari, S. H. (2015). Relation between parvovirus B19 infection and fetal mortality and spontaneous abortion. *Medical journal of the Islamic Republic of Iran*, 29, 197.
9. Gao, Y.-L., Gao, Z., He, M., Liao, P. (2018). Infection status of human parvovirus B19, cytomegalovirus and herpes simplex Virus-1/2 in women with first-trimester spontaneous abortions in Chongqing, China. *Virology Journal*, 15 (1). doi: <http://doi.org/10.1186/s12985-018-0988-5>
10. Habibzadeh, S., Peeri-Doghaheh, H., Mohammad-Shahi, J., Mobini, E., Shahbazzadegan, S. (2016). The prevalence of parvovirus B19 infection among pregnant women of Ardabil in 2013. *Iranian journal of microbiology*, 8 (3), 214–218.
11. Delwart, E. (2012). Human parvovirus 4 in the blood supply and transmission by pooled plasma-derived clotting factors: does it matter? *Transfusion*, 52 (7), 1398–1403. doi: <http://doi.org/10.1111/j.1537-2995.2012.03721.x>
12. Lamont, R., Sobel, J., Vaisbuch, E., Kusanovic, J., Mazaki-Tovi, S., Kim, S. et. al. (2010). Parvovirus B19 infection in human pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology*, 118 (2), 175–186. doi: <http://doi.org/10.1111/j.1471-0528.2010.02749.x>
13. Ling, Y., Yu, Y.-H., Jin, S., Hu, C.-X., Peng, W., Zhang, H.-Y. et. al. (2017). Study of concentration of amniotic fluid alpha-fetal protein in thalassemia fetus. *Asian Pacific Journal of Tropical Medicine*, 10 (2), 201–203. doi: <http://doi.org/10.1016/j.apjtm.2017.01.001>